CLINICAL RESEARCH

Superior Graft-versus-Leukemia Effect Associated with Transplantation of Haploidentical Compared with HLA-Identical Sibling Donor Grafts for High-Risk Acute Leukemia: An Historic Comparison

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The outcomes of an historic comparison of 117 consecutive, high-risk, acute leukemia patients undergoing hematopoietic stem cell transplantation (HSCT) from HLA-mismatched/haploidentical donors (HID, n = 81) or HLA-identical sibling donors (ISD, n = 36) without the use of in vitro T cell depletion (TCD), between the period of January 2005 and April 2009 were compared. Full engraftment was achieved in 98% of patients in the HID group and 97% in the ISD group. The cumulative incidences of grades II–IV acute graft-versus-host disease (aGVHD) in the HID and ISD cohorts were 49% and 24%, respectively ($P = .014$) with a relative risk (RR) of 2.99 (1.25-7.21) ($P = .014$). The incidence of chronic GVHD (cGVHD) did not differ significantly between the 2 cohorts. The 2-year cumulative incidence of relapse was significantly lower in HID (26%) than in ISD patients (49%) ($P = .008$). The 2-year cumulative incidence of nonrelapse mortality (NRM) was comparable in recipients of HID (34%) and ISD grafts (38%) ($P = .85$). The 3-year probability of overall survival (OS) was higher in HID patients (42%) than in ISD (20%) ($P = .048$) patients. Our comparisons suggest that HID transplants can achieve a stronger graft-versus-leukemia (GVL) effect than ISD for high-risk acute leukemia patients.

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KEY WORDS: Hematopoietic stem cell transplantation, HLA, Mismatched

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is 1 of the best, and sometimes the only, option for the treatment of leukemia, particularly for patients with high-risk leukemia. It is widely known that the relapse rate of allogeneic HSCT (allo-HSCT) is lower than that of auto- or syngeneic HSCT. Immune cells derived from the donor contribute to the eradication of leukemia after allo-HSCT, whereas auto- or syngeneic HSCT have no graft-versus-leukemia (GVL) effect. The GVL effect is usually identified in retrospective analyses of relapse rates following HSCT from human leukocyte antigen (HLA)-identical sibling donors for leukemia [1] and has subsequently been extensively confirmed in other transplant settings. Kanda et al. [2] reported that the incidence of relapse was dramatically decreased with 1-locus–mismatched family member HSCT compared to matched HSCT for high-risk diseases (19% versus 47%; $P = .004$). Reports from IBMTR also showed that in acute leukemia, relapse risk was lower after alternative-donor compared with HLA-identical sibling transplants. This difference was statistically significant ($P < .05$) for 2-HLA-antigen–mismatched related and HLA-antigen–mismatched unrelated donors [3].

Great progress has been made in haploidentical donor (HID) HSCT over the past 20 years, and it has become a feasible option for leukemia patients especially with high-risk features without a HLA-identical sibling donor (ISD). It has been speculated that HID HSCT may potentially exert a strong GVL effect. However, comparative clinical studies to confirm the potential beneficial GVL effects are lacking. One possible reason for the lack of such studies is that most HID HSCT is performed with an in vitro T cell depletion (TCD) modality [4-13]. The lack of a rapid donor T cell recovery secondary to profound graft TCD limits the overall antileukemia potential of the TCD modality compared with alternative T cell–replete options.

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Recently, we developed a new method for HLA-mismatched/haploidentical transplantation without in vitro TCD. The strategy comprises sequential, in vivo modulation of the recipient and donor T cell functions and the determination of the dose of donor hematopoietic stem cells using the GIAC protocol (G, donor treatment with recombinant granulocyte colony-stimulating factor [rhG-CSF]; I, intensified immunological suppression; A, antihuman thymocyte immunoglobulin [ATG] for the prevention of graft-versus-host disease [GVHD]; C, combination of peripheral blood stem cell transplantation [PBSCT], and bone marrow transplantation [BMT]) [14]. Using this protocol, promising results for HID HSCT without in vitro TCD have been achieved at our institute [14-16]. Whether HID HSCT has a stronger GVL effect is under consideration under our new transplant protocol. The purpose of this nonrandomized, single-center, retrospective study was to comparatively analyze transplantation outcomes in a consecutive series of high-risk acute leukemia patients who underwent HSCT from either HID or ISD without in vitro TCD at our institute.

MATERIALS AND METHODS

Patient Eligibility

Consecutive patients with high-risk acute leukemia (n = 117) receiving HSCT from either ISD (n = 36) or HID (n = 81) between January 2005 and April 2009 were enrolled. Patients receiving HSCT as a second transplant following relapse after a first auto- or allogeneic transplantation were excluded. Thirty of the 81 high-risk acute leukemia patients from the HID cohort were previously reported in 2009 [16], and 19 of those 30 patients were previously reported in another report in 2009 as well [15]; additionally, 1 of those 19 patients was previously reported in 2006 [14]. All these patients reported before were enrolled and further followed in this study. The protocols were approved by the institutional review board of the Peking University Institute of Hematology, and all patients and their donors signed consent forms. Patients in complete remission (CR)3 or beyond (patients in CR2 were classified as intermediate risk in most studies), nonremission, or CR1 with poor-risk cytogenetic abnormalities were classified as high risk. For patients with acute myelogenous leukemia (AML), poor-risk cytogenetic abnormalities included t(9;22) + other, –5 or del(5) + other, –7 or del(7) only, –7 or del(7) + other, del(11) only, del(11) + other [17]. For patients with acute lymphoblastic leukemia (ALL), poor-risk cytogenetic abnormalities included t(4;11), t(9;22), or t(8;14). A small proportion of patients were evaluated for the Flt3 internal tandem duplication (patients undergoing HSCT after year 2008), so this part of the information was not provided in this study. Characteristics of the patients and donors are summarized in Table 1.

Donor Selection

A human leukocyte antigen (HLA)-matched sibling donor was the first choice for allo-HSCT. If a matched sibling donor was unavailable as a first treatment option, patients without a suitable closely HLA-matched unrelated donor, that is, with more than 8 of 10 matching HLA-A, B, C, DR, and DQ loci and at least 5 of 6 matching HLA-A, B, and DR loci, or whose disease status left insufficient time for an unrelated donor search, were eligible for HID HSCT. To determine HLA-A and HLA-B status, low-resolution DNA techniques were used. High-resolution techniques were used for HLA-DRB1 typing. All donor-recipient pairs were typed at the HLA-A, B, and DR loci at our institute. Patients who had undergone an unrelated donor search were typed at the HLA-A, B, C, DR, and DQ loci in China at an unrelated marrow bank. Thirty-six sibling donor-recipient pairs were fully HLA matched at the HLA-A, B, and DR loci. For the other 81 donor-recipient pairs, each patient received stem cells from a family member who shared 1 HLA haplotype with the patient but differed to a variable degree for the HLA-A, B, and D antigens of the haplotype not shared. Apart from each donor-recipient pair, HLA typing was done for parents and offspring to be strictly analyzed to guarantee true haploid genetic background. The HLA disparities in the mismatched cohort are shown in Table 1.

Conditioning Regimen

The conditioning therapy for the HID group was modified BUCY2 plus ATG (thymoglobulin) consisting of cytarabine (4 g/m^2/day) intravenously on days –10 to –9; busulfan (4 mg/kg/day) orally on days –8 to –6 before January 2008, and busulfan (3.2 mg/kg/day) intravenously on days –8 to –6 after January 2008; cyclophosphamide (1.8 g/m^2/day) intravenously on days –5 to –4; Me-CCNU (250 mg/m^2) orally once on day –3; and ATG (thymoglobulin, 2.5 mg/kg/day; Sang Stat, Lyon, France) intravenously on days –5 to –2. Patients in the ISD group received hydroxycarbamide (80 mg/kg) orally on day –10 and a lower dose of cytarabine (2 g/m^2/day) on day –9, but otherwise, an identical regimen to the HLA-mismatched patients without ATG.

GVHD Prophylaxis

All patients received cyclosporine A (CSA), mycophenolate mofetil (MMF), and short-term methotrexate (MTX) for GVHD prophylaxis [15]. On day +1, MTX (15 mg/m^2) was administered intravenously followed by administration at 10 mg/m^2 on days +3 and

Table 1. Characteristics of Patients and Grafts

Characteristics	ISD n = 36	HID n = 81	P *
Age, years, median (range)	41 (15-56)	29 (5-50)	.001
Gender, no. (%)			.25
Male	27 (75)	52 (64)	
Female	9 (25)	29 (36)	
Diagnosis, no. (%)			.12
Acute myeloid leukemia	20 (56)	30 (37)	
CR3 or beyond	5	2	
Nonremission	15	28	
Acute lymphoid leukemia	16 (44)	51 (63)	
PH negative	3 (8)	16 (20)	
CR3 or beyond	2	0	
Nonremission	1	16	
PH positive	13 (36)	35 (43)	
Duration from diagnosis to HSCT, mo			.66
Median (range)	6.0 (2.5-180)	6.3 (1-219)	
Donor-patient sex matched, no. (%)			.65
FM	10 (28)	24 (30)	
FF	6 (17)	20 (24)	
MM	17 (47)	29 (36)	
MF	3 (8)	8 (10)	
No. of HLA-A,-B,-DR mismatched, no. (%)			—
0	36 (100)	0	
1		7 (8)	
2		20 (25)	
3		54 (67)	
Graft type, no. (%)			.001
Bone marrow + peripheral blood cell	19 (53)	71 (88)	
Peripheral blood cell	17 (47)	10 (12)	
Median MNCs, $\times 10^8$/kg (range)	6.8 (4.0-14)	7.1 (3.6-11)	.11
Median $CD34^+$ count, $\times 10^6$/kg (range)	2.5 (1.1-3.6)	2.9 (1.5-6.2)	.61
Median $CD3^+$ count, $\times 10^8$/kg (range)	1.9 (0.7-3.9)	1.8 (0.9-7.4)	.71
Follow-up time, months			.21
Median (range)	11 (0.6-46)	16 (0.8-59)	
Follow-up time in survivors, months			.98
No. of evaluable patients	10	41	
Median (range)	23 (10-46)	22 (11-59)	

MNC indicates mononuclear cell; HSCT, hematopoietic stem cell transplantation; ISD, identical sibling donors; HID, haploidentical donors; CR, complete remission; —, data not comparable.

*The chi-square test was used for categorical variables; the Mann-Whitney *U*-test was used for continuous variables.

+6 after ISD or HID HSCT and additionally on day +11 after HID HSCT. MMF was discontinued upon engraftment after ISD HSCT, whereas in HID patients, MMF was tapered from 1 g/day to 0.5 g/day on day 30 and was discontinued over days 45 to 60 based on the presence or absence of severe GVHD, infectious diseases, and relapse risk.

Collection of Hematopoietic Cells

Donors were primed with rhG-CSF (Filgrastim, Kirin, Japan; 5 μg/kg per day) injected subcutaneously for 5 to 6 consecutive days. On the fourth day, bone marrow (G-BM) cells were harvested. The target mononuclear cell (MNC) count was $2 \times 10^8 \cdot kg^{-1}$ of the recipient weight. In instances of major ABO blood group incompatibility, red blood cells were removed from bone marrow cells by density gradient sedimentation with Hespan (B. Braun Medical Inc, Irvine, CA), according to the manufacturer's instructions. On the fifth day (and sixth day if needed, that is, if target MNC was not reached on the fifth day), peripheral blood cells (G-PB) were collected with a COBE Blood Cell Separator (Spectra LRS, COBE BCT, Inc., Lakewood, CO) at a rate of 80 mL/min from a total blood volume of 10 L. The target MNC count was $3 \times 10^8 \cdot kg^{-1}$ of the recipient weight. Patients who received only G-PB received 2 days of leukapheresis collections from their donors on the fourth and fifth days. The target MNC count was $5 \times 10^8 \cdot kg^{-1}$ of the recipient weight. Greater than 6×10^8 MNC/kg or 4×10^6 $CD34^+$ cells/kg were planned for harvest. The extra harvested cells were cryopreserved with dimethyl sulfoxide in a nitrogen tank. Data on the composition of grafts are shown in Table 1.

Prevention and Treatment of Relapse

Modified donor lymphocyte infusion (DLI) was planned from days 45 to 120 after transplantation in patients when no GVHD occurred or, if CSA was tapered or stopped, GVHD was controlled. Before DLI, serious infection must be cleared and no serious organ failure can be present. The modified DLI regimen comprised G-CSF-primed PBSCs instead of harvested nonprimed donor lymphocytes and short-term immunosuppressive agents [18]. Lymphocytes were obtained from cryo-preserved G-CSF mobilized

peripheral blood (G-PB) (described previously in the section entitled "collection of hematopoietic cells"). For patients receiving DLI before day 90 posttransplantation, the original CSA treatment was continued for another 2 weeks after the infusion, and then tapered and discontinued within 4 weeks if no DLI-associated GVHD occurred. For patients receiving DLI after day 90, immunosuppression was discontinued for a minimum of 2 weeks and, if no active GVHD was present, before DLI. These patients took oral CSA or methotrexate (MTX, at a dose of 10 mg, repeated at day 8 after the first dose and then at a weekly interval), for 2 to 4 weeks after DLI for the prevention of DLI-associated GVHD. The median number of $CD34^+$ cells infused was 0.59 (0.05-2.2) $\times$ 10^6/kg. The median number of $CD3^+$ cells infused was 0.55 (0.3-1.5) $\times$ 10^8/kg. Twenty-one patients (26%) in the HID group and 17 patients (47%) in the ISD group received prophylactic DLI. Reasons for not giving prophylactic DLI in the HID cohort were as follows: graft failure (n = 2), GVHD (n = 9), infection not controlled within 120 days (n = 3), early relapse (n = 4), death within 45 days (n = 3), disagreement (n = 9), and the remaining 30 were Ph+-ALL patients. Reasons for not giving prophylactic DLI in the ISD cohort were as follows: GVHD (n = 3), infection (n = 2), early relapse (n = 3), early death (n = 1), and the remaining 10 were PH+-ALL patients.

When hematologic or cytogenetic relapse was diagnosed after HCT, the relapse was treated with a trial phase of immunosuppressant withdrawal followed by therapeutic DLI. Patients whose blast count in the bone marrow at the time of post-HSCT relapse was over 20% had received prior chemotherapy [19]. DLI was given 48 hous after the last chemotherapy dose. For patients whose blast count was less than 20%, DLI was given without chemotherapy after immunosuppression had been discontinued for a minimum of 2 weeks and no evidence of active GVHD was present. G-CSF-primed peripheral blood stem cells (GPBSC) were used instead of steady donor lymphocyte harvests [20]. The median number of infused $CD34^+$ cells was 2.69 (0.82-9.69) $\times$ 10^6/kg. The median number of infused $CD3^+$ cells was 2.09 (0.84-5.6) $\times$ 10^8/kg. All patients received short-term immunosuppressive agents for 2 to 4 weeks for the prevention of DLI-associated GVHD.

Therapy of DLI-Mediated GVHD

For acute GVHD (aGVHD) after MTX or CSA as prophylaxis regimens, methylprednisolone (MP) is the best initial therapy. Prednisone and CSA are considered as the first-line therapy for patients with chronic GVHD (cGVHD). Other therapeutic options for aGVHD or cGVHD are MMF, tacrolimus (FK506), azathioprine, thalidomide, monoclonal antibodies directed against CD3 and CD25, and MTX.

Imatinib mesylate was planned to be administered to Ph+-ALL patients without serious infection, GVHD, or organ failure from engraftment for 3 to 6 months if the absolute neutrophil count (ANC) remained above 1.0 $\times$ 10^9/L and the platelet count remained above 50 $\times$ 10^9/L before administration. Daily dosing of imatinib was initiated at 400 mg/m^2 for adults or 260 mg/m^2 for children. Nineteen patients in the HID group (54% of Ph+-ALL patients) and 7 patients in the ISD group (54% of Ph+-ALL patients) received prophylactic imatinib mesylate. Reasons for not giving prophylactic imatinib in the HID cohort were as follows: GVHD (n = 4), infection not controlled within 120 days (n = 4), death within 45 days (n = 3), and disagreement (n = 6). Reasons for not giving prophylactic imatinib in the ISD cohort were as follows: graft failure (n = 1), GVHD (n = 1), infection not controlled within 120 days (n = 3), and disagreement (n = 1).

Ph+-ALL patients who experienced a cytogenetic or hematologic relapse after HSCT and patients who demonstrated rising levels (1 log increase) of bcr/abl RNA transcripts by real-time quantitative PCR (RQ-PCR) were given Imatinib for therapeutic use.

Definitions and Assessments

Neutrophil engraftment was defined as an ANC of 0.5 $\times$ 10^9/L or more for 3 consecutive days, and platelet engraftment was defined as 20 $\times$ 10^9/L or more for 7 consecutive days without transfusion. Primary engraftment failure was defined as the absence of donor-derived myeloid cells at day 60 in patients surviving beyond day 28 after transplantation or as the need for a second allogeneic transplant or reconstitution with autologous cells. Chimerism was determined by at least 2 of the following 3 methods: DNA-based HLA typing, polymerase chain reaction (PCR) DNA fingerprinting of short tandem repeats on recipient PB cells, and chromosomal fluorescence in situ hybridization (FISH) on recipient BM cells. Acute GVHD and cGVHD were defined according to published criteria [21,22]. Relapse was defined by morphologic evidence of disease in the peripheral blood, marrow, or extramedullary sites or by the recurrence and sustained presence of pretransplantation chromosomal abnormalities. Patients showing MRD (eg, the presence of bcr/abl RNA transcripts by PCR) were not classified as having relapsed. Leukemia-free survival (LFS) was defined as survival in CR at last follow-up.

Statistical Analysis

Cumulative incidences were estimated for engraftment, GVHD, nonrelapse mortality (NRM), and relapse to accommodate competing risks. Competing risks for engraftment was death without engraftment;

competing risks for GVHD was death without GVHD, relapse, and graft rejection; relapse was a competing risk for NRM, and NRM was a competing risk for relapse. For all of our study population (with or without DLI), time to GVHD was defined as the time from HSCT to the onset of any grade of GVHD; aGVHD was censored at day 100 after HSCT and cGVHD was censored at last follow-up. Associations between graft type and outcome were evaluated using an add-on package for the R statistical software, which allows for the estimation of the semiparametric proportional hazards model for the subdistribution of a competing risk analysis as proposed by Fine and Gray [23]. In addition to the hematopoietic stem cell source, the following variables were considered as covariates: recipient age, recipient and donor sex, degree of ABO matching, graft source, disease type, time from diagnosis to transplantation, and dose of nucleated cells infused. When groups were compared according to continuous covariates, Mann-Whitney *U*-tests were used. A chi-square test was used to compare categorical covariates. The probability of survival was calculated using the Kaplan-Meier method. To detect possible influences of allogeneic transplantation on leukemia relapse in association with and independent of GVHD (acute or chronic), patients with engraftment were categorized into 2 groups: with aGVHD or without aGVHD (until day 100 after HSCT or until last follow-up for patients not surviving beyond day 100 after HSCT). Patients that survived longer than 100 days after HSCT were categorized into 2 groups: with cGVHD or without cGVHD (until final follow-up). For each group, associations between graft type and leukemia relapse were evaluated using cumulative incidences to accommodate competing risks for NRM. The 95% confidence interval (95% CI) was calculated to compute the standard error. SAS version 8.2 (SAS Institute, Cary, NC) and S Plus 2000 (Mathsoft, Seattle, WA) were used for most analyses. Endpoints were calculated at last contact, with the date of the latest follow-up being March 1, 2010.

RESULTS

Engraftment

After myeloid recovery, 2 patients died before day 28 posttransplantation. Among the patients surviving beyond day 28, analyses of chimerism indicated that 78 out of 80 (98%) patients in the HID group and 34 out of 35 (97%) patients in the ISD group achieved full donor chimerism by day 30 after HSCT. The cumulative 30-day myeloid engraftment probabilities were 98.7% in the HID cohort and 97.2% in the ISD group ($P = .08$). Patients engrafted to absolute neutrophil counts exceeding 0.5×10^9/L in a median time of 13 days (range: 9-29 days) in the HID group and 16 days (range: 11-25 days) in the ISD group ($P = .061$).

Three patients (2 HID, another ISD) had primary graft failure. All 3 received G-PB as the graft source. One HID patient received a second HSCT with G-BM + G-PB from the same donor on day 29 after conditioning with cyclophosphamide (CTX 1000 mg/day × 2), fludarabine (fludarabine 50 mg/day × 4), and anti-CD25 monoclonal antibodies (MoAb). Despite the peak white blood cell count (WBC) of 0.9×10^9/L on day 32 after the second HSCT, no sustained myeloid engraftment was confirmed until he died of sepsis on day 41 after the second HSCT. The other HID patient experienced graft failure on day 17 after 2 days of transient ANC $>0.5 \times 10^9$/L. She received G-BM + G-PB cells from the same donor after anti-CD25 MoAb preparation and achieved sustain engraftment again on day 12 after the second HSCT. The ISD patient gave up treatment on day 28 without engraftment and died of severe infection on day 40 after transplantation. No patients had secondary graft failure.

Seventy-three (90%) and 31 (86%) patients achieved platelet engraftments in the HID and ISD groups, respectively. The 50-day cumulative platelet engraftment probability was 86% in the HID cohort and 83% in the ISD group ($P = .66$). These platelet engraftments occurred at 15 days (range: 7-74 days) and 15 days (range: 7-100 days) for the HID and ISD patients, respectively ($P = .66$).

GVHD

At day 100 after transplantation, the cumulative incidences of grade II–IV aGVHD in the HID and ISD cohorts were 49% (CI, 37%-61%) and 24% (CI, 9%-41%), respectively ($P = .014$; Figure 1A), with a relative risk (RR) of 2.99 (1.25-7.21) ($P = .014$; Table 2). The cumulative incidences of grade III–IV aGVHD in the HID and ISD cohorts were 15% (CI, 5%-25%) and 4% (CI, 1%-7%), respectively ($P = .13$).

Sixty-nine patients in the HID cohort and 28 patients in the ISD cohort survived longer than 100 days after HSCT and were eligible for evaluation of cGVHD. The 2-year cumulative incidences of cGVHD in the HID and ISD cohorts were 62% (CI, 49%-75%) and 39% (CI, 20%-58%), respectively ($P = .11$; Figure 1B), with an RR of 1.52 (0.69-3.34) ($P = .30$; Table 2).

Relapse

At last follow-up with a median of 12.3 months, 18 patients (22%) in the HID group and 17 patients (47%) in the ISD group experienced leukemia relapse. Two-year incidences of relapse for the HID and ISD HSCT groups were 26% (15%-37%) and 49% (31%-67%), respectively ($P = .008$; Figure 2A), with

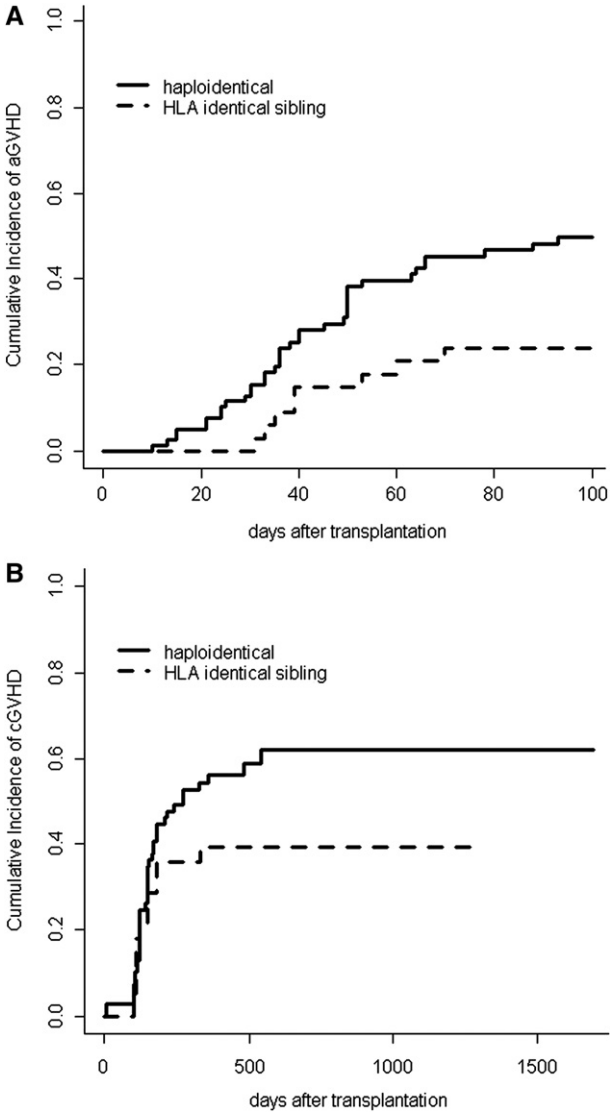

Figure 1. Cumulative incidence of aGVHD (A) and cGVHD (B) after ISD or HID HSCT ($P = .014$ and $P = .11$, respectively).

an RR = 0.21 (95% CI, 0.08-0.53) ($P = .001$; Table 2). In multivariate analysis, age was not a significant factor influencing relapse rate, with a RR = 0.99 ($P = .57$). In patients with aGVHD, the relapse rates for the HID and ISD HSCT groups were 23% and 13%, respectively ($P = .75$). In patients without aGVHD, the relapse rate for the HID and ISD HSCT groups were 26% and 59%, respectively ($P = .002$). In patients with cGVHD, the relapse rate for the HID and ISD HSCT groups were 22% and 36%, respectively ($P = .23$). In patients without cGVHD, the relapse rate for the HID and ISD HSCT groups were 22% and 52%, respectively ($P = .027$).

Eight of 30 patients with AML, 7 out of 16 with PH-negative ALL, and 3 out of 35 with PH-positive ALL relapsed after HID HSCT. Thirteen out of 20 with AML, 2 out of 3 with PH-negative ALL, and 2 out of 13 with PH-positive ALL relapsed after ISD HSCT.

Table 2. Multivariate Analysis of aGVHD, cGVHD, TRM, Relapse, and Survival

Outcome	Hazard ratio (95% CI)*	P
Myeloid engraftment		
HID versus ISD	1.19 (0.75-1.91)	.46
Platelet engraftment		
HID versus ISD	0.73 (0.43-1.23)	.23
Acute graft-versus-host disease		
HID versus ISD	2.99 (1.25-7.21)	.014
Other significant risk factors		
ABO blood group		.023†
Matched	1.00	
Minor mismatched	0.88 (0.39-1.97)	.75
Major mismatched	2.11 (1.17-3.80)	.012
Chronic graft-versus-host disease		
HID versus ISD	1.52 (0.69-3.34)	.30
Nonrrelapse-related mortality		
HID versus ISD	1.93 (0.85-4.37)	.12
Relapse		
HID versus ISD	0.21 (0.08-0.53)	.001
Other significant risk factors		
Diagnosis		.002†
PH+ acute lymphoid leukemia	1.00	
PH− acute lymphoid leukemia	8.11 (2.38-27.63)	.001
acute myelogenous leukemia	4.94 (1.81-13.52)	.002
Overall survival		
HID versus ISD	0.71 (0.37-1.36)	.30

CI indicates confidence interval; ISD, identical sibling donors; HID, haploidentical donors.
*The hazard ratio is for HID transplantation compared with ISD transplantation.
†Two degrees of freedom test.

Five patients received imatinib mesylate for treatment of cytogenetic or hematologic relapse including 3 in the HID group and 2 in the ISD group. At the time of last follow-up, 27 patients had died of relapse including 14 in the HID group and 13 in the ISD group, with a median time to death of 245 days (range: 34-790) and 284 days (range: 53-727) after HSCT, respectively.

NRM

Two patients died within 28 days of HSCT, 1 in the ISD group at day 18 because of heart failure, and another in the HID group at day 24 because of infection. Analyses of NRM are described in Table 3. Two-year incidences of NRM for the HID and ISD HCT groups were 34% (22%-46%) and 38% (21%-55%), respectively ($P = .85$; Figure 2B), with an RR = 1.93 (CI, 0.85-4.37) ($P = .12$; Table 2). Nine patients (4 with DLI, 5 without DLI) died beyond 1 year after HSCT. Among the 9 patients, 3 patients in the ISD group died at days 404, 524, and 572 because of infection. In the HID group, 3 died at days 367, 478, and 690 from infection, 2 died at day 467 and 764 from GVHD, and 1 died at day 551 from organ failure.

Long-Term Follow-Up and Survival

The 3-year overall survival (OS) after HCT was 42% (CI, 30%-54%) for HID patients and 20% (CI, 4%-36%) for ISD patients ($P = .048$). The 3-year

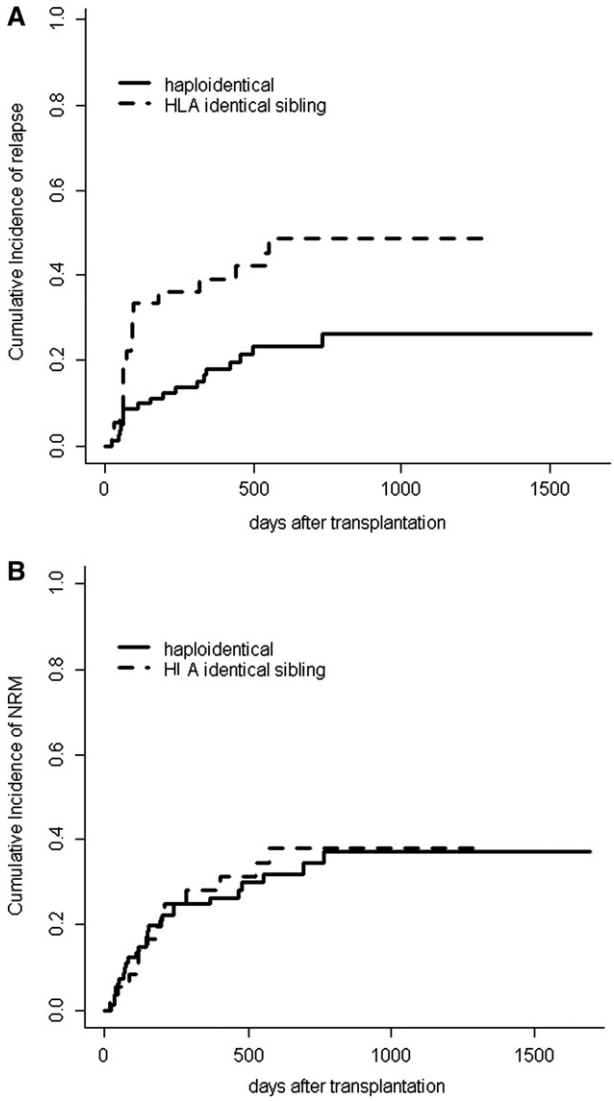

Figure 2. Cumulative incidence of relapse (A) and NRM (B) after ISD or HID HSCT ($P = .008$ and $P = .85$, respectively).

LFS in the HID group was 42% (30%-54%) versus 15% in the ISD group (CI, 1%-29%) ($P = .029$; Figure 3A-B).

DISCUSSION

Some studies have shown that HLA disparity might have a stronger GVL effect than ISD HSCT [2,24]; however, conflicting data exist regarding this issue [25,26]. Kanda et al. [2] reported that the cumulative incidence of relapse was 22% after matched HSCT for standard-risk diseases, which was not significantly different from that after 1-locus–mismatched HSCT (15%; $P = .25$). In contrast, the incidence was 47% versus 19% ($P = .004$) for high-risk diseases. Beatty et al. [24] reported that there was a trend for the probability of relapse to be greater in the matched unrelated group (23%) than in the partially matched unrelated group (12%). Unrelated donor HSCT has comparable or lower relapse rate than ISD HSCT [3,25]. Data from the International Bone Marrow Transplant Registry (IBMTR) report showed that 3-year probability of relapse was 13% after 1-antigen–mismatched unrelated HSCT for intermediate-stage diseases, which was not significantly different from that after ISD HSCT (29%; $P = .10$). In contrast, the incidence was 20% versus 73% ($P = .03$) for advanced-stage diseases [3]. The present data show that the relapse rate for high-risk patients after HID HSCT was lower than with ISD HSCT. Data from our recent comparison between haploidentical and unrelated HSCT did not indicate a significant difference for high-risk acute leukemia patients because the small population of high-risk patients in the unrelated group (18 patients) made it difficult to demonstrate the difference. However, the 2-year probability of relapse for standard-risk patients was 8% versus 19% ($P = .033$) in the haploidentical and unrelated cohorts [16]. These observations indicate that HID HSCT might have a stronger GVL effect than ISD HSCT.

Table 3. Causes of Death

Causes of Death	ISD, n = 26	HID, n = 40
Relapse	13 (50)	14 (35)
Infection	10 (38)	22 (55)
Bacteria	4	9
Fungal	3	8
Viral	2	3
Unknown	1	2
GVHD	1 (4)	3 (7)
Organ failure	2 (8)	1 (3)

GVHD indicates graft-versus-host disease; ISD, identical sibling donors; HID, haploidentical donors.

Many factors can influence GVL and the relapse rate after HSCT, such as disease type and remission status before HSCT, T cell number infused, conditioning regimen, GVHD prophylaxis, use of imatinib, presence of aGVHD and cGVHD, patient age, and other factors.

The composition of disease type was comparable between the 2 study groups, with the exception that the HID cohort comprised a larger proportion of ALL patients (see Table 1). The GVL effect was generally weaker for ALL patients; however, in a larger proportion of ALL patients in the HID cohort compared to the ISD group, the relapse rate was still lower for HID patients. For PH+-ALL patients, the successful pre- or posttransplantation use of imatinib [27,28] may reduce the incidence of relapse after allo-HCT. However, in the current study, the proportion of PH+-ALL patients (see Table 1) and the proportion of patients receiving prophylactic imatinib ($P = .98$) in the 2 cohorts were comparable.

All patients in this study were treated with similar conditioning regimens without TCD. The 1 disparity

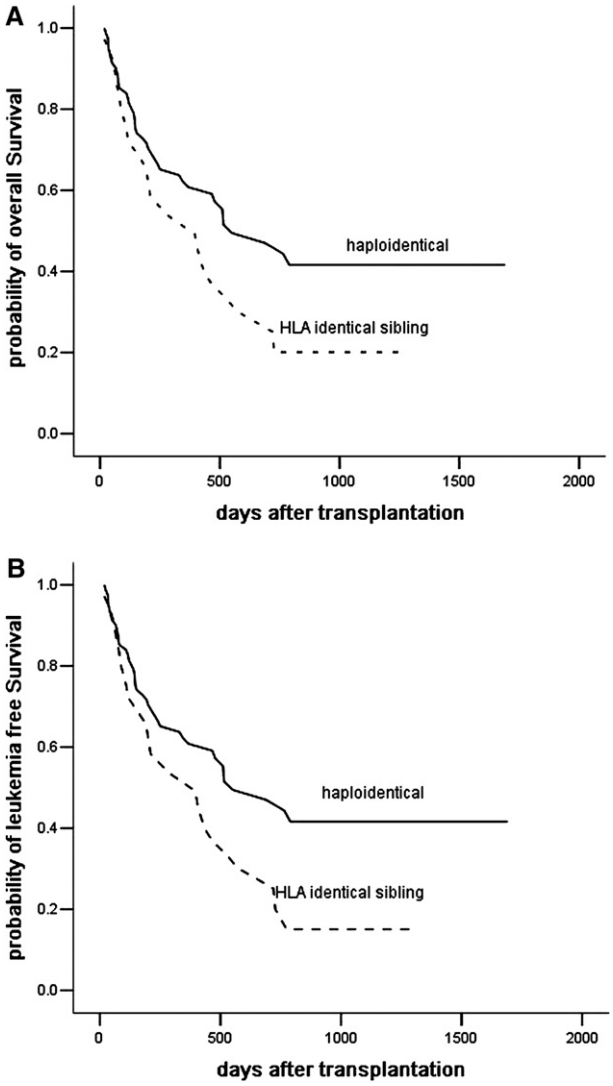

Figure 3. Probability of OS (A) and LFS (B) after ISD or HID HSCT ($P = .048$ and $P = .029$, respectively).

in the GVHD prophylaxis schedule was that HID patients received ATG for additional immunosuppression. Although this is a distinguishing feature between the groups, we do not believe that this affects the interpretation of the data or the conclusions of the study. The requirement for more intense immunosuppression in the haploidentical group is an integral aspect of the current treatment regimen for these patients to prevent GVHD and to facilitate engraftment [4]. It is unlikely that patients would be enrolled in a study with an equivalent degree of immunosuppression between recipients of matched related grafts and recipients of haploidentical grafts. Additionally, the use of ATG to prevent GVHD without influencing relapse [29,30] may make the GVL effect even weaker [31]. Remberger [31] reported that the probabilities of relapse were 41% and 27% with or without ATG as part of conditioning after unrelated HSCT. In patients with CML in chronic phase (CP), the relapse probability was significantly higher in the ATG group (60%) compared to the non-ATG cohort (18%; $P = 0.04$). Another disparity was that HID patients received a higher dose of cytosine arabinoside (Ara-c). It is unlikely that the lower relapse rate in the HID group was attributed to the higher dose of Ara-c; however, this variable cannot be completely excluded.

Numerous studies, including our own study, showed that the presence of cGVHD is more important than that of aGVHD for the GVL effect [18,25,32]. In the current study, a higher incidence of aGVHD occurred in haploidentical than in ISD HSCT; however, in patients with aGVHD, the relapse rates for the HID and ISD HSCT groups were comparable ($P = .75$). In patients without aGVHD, the relapse rates for the HID and ISD HSCT groups were 26% and 59% ($P = .002$), whereas in patients with cGVHD, the relapse rates for the HID and ISD HSCT groups were comparable ($P = .23$). In patients without cGVHD, the relapse rates for the HID and ISD HSCT groups were 22% and 52% ($P = .027$). This suggested a GVL effect in the absence of GVHD, as confirmed by HLA-identical sibling transplants without GVHD having a reduced relapse risk compared with syngeneic transplants [32].

Because of the 1-child policy in China, more than half of our haploidentical donors were parents; thus, an age difference existed between our 2 study groups (see Table 1). Although younger patients tend to have biologically less aggressive leukemia, our study populations were all high-risk patients, and in multivariate analysis, age was not a significant factor affecting relapse rate.

Although these potentially confounding factors may play a role in the GVL effect, we believe that under our general protocol, the lower incidence of relapse in the HID group cannot be explained exclusively by any of the factors discussed. Our data suggest a superior antileukemia effect of HID without TCD compared to ISD HSCT, at least for high-risk acute leukemia patients. However, the biologic mechanism needs to be further explored.

The engraftment rate and NRM were comparable between the 2 study cohorts. In multivariate analysis, there was a trend toward a higher risk of NRM among recipients of haploidentical transplants compared to ISD transplants (hazard ratio [HR] 1.93, $P = .12$), whereas NRM was actually slightly lower among recipients of haploidentical transplants. This result may arise from the age difference between the 2 groups because age was a significant factor affecting NRM in multivariate analysis (data not shown). In a previous comparative study reported by Lu et al. [33], NRM for patients was comparable for ISD and HID patients, with a 2-year NRM of 14% and 22%, respectively. NRM in the current study was higher than in that

previous report because of the high-risk disease status in the current report. The proportion of high-risk patients in that previous report was 16% and 24% for ISD and HID cohorts. Many studies, including our own showed, that disease status can dramatically affect NRM [3,14,15,34]. Data from the IBMTR report showed that the 3-year NRM was 21%, 38%, and 54%, respectively, for early-, intermediate-, and advanced-stage diseases after ISD HSCT [3]. MacMillan et al. [34] reported that 1 of the factors associated with significantly worse 2-year TRM was a high-risk diagnosis at the time of HCT (RR, 1.36; 95% CI, 1.07-1.73; $P < .01$). In our 2006 report, the 2-year NRM for haploidentical patients was 19.5% and 31.1%, respectively (similar to the current report), in the standard-risk group and in the high-risk group [14]. In our 2009 report, the 2-year NRM for haploidentical acute leukemia patients in standard-risk and high-risk groups was 18.6% and 30% for AML and 18.2% and 63.3% for ALL, respectively [15]. The sets of transplanted patients between the current report (from January 2005 to April 2009) and the previous report by Lu et al. (from January 2002 to July 2004) were not overlapping, but the difference in the NRM between the 2 studies cannot be explained by the transplant year difference. Data from another comparative study conducted by our group between January 2004 and December 2007 showed that the NRM was similar to the previous report by Lu et al. (20% and 18% after HID and unrelated HSCT) [16]. Most of the sets of transplanted patients between the previous report by Lu et al. and that comparative study reported in 2009 were not overlapping, and the proportion of high-risk patients was similar. NRM appears to have occurred much later in the current report than in the previous report by Lu et al. (Figure 2B). The primary cause for the delayed NRM is described in the NRM Results section stating that DLI may play a role for the delayed NRM.

A higher probability of survival was achieved for HID patients in the current report. In the previous comparative study reported by Lu et al. [33], survival for patients with advanced disease was comparable for ISD and HID patients, with 2-year probabilities of survival of 45% and 47%, respectively. It appears that the LFS in ISD patients in the current study was inferior to that in the previous report. However, it must be noted that previously, a smaller study population (25 ISD and 32 HID patients), a different definition of high risk (apart from advanced or resistant AL, also including myelodysplastic syndrome-refractory anemia with excess blasts (MDS-RAEB) and chronic myeloid leukemia-blastic phase (CML-BP), a heterogeneous series of patients (with a variety of diagnoses, including MDS and CML), and shorter follow-up time for survival may have contributed to its better outcomes. By contrast, the outcome of HLA-matched transplantation in high-risk patients has remained fairly constant at approximately 20% survival in many other larger studies [2,3,25,29,35].

Considering the significantly lower relapse rate and higher survival probability for HID patients than for ISD patients, whether HID HSCT with ex vitro T cell depletion should be selected instead of ISD donors in patients with high-risk leukemia deserves further evaluation.

In conclusion, the current study showed that a lower relapse rate, a similar engraftment rate, and a higher survival probability was achieved with HID patients than with ISD patients. The results suggest that HID HSCT might achieve a better antileukemia effect for high-risk acute leukemia patients.

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AUTHORSHIP STATEMENT

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